Studies on Phytochemical Screening and Estimation of Antimicrobial Activity of Medicinal Plant Extract *Achyranthes aspera* Linn.

SYNOPSIS

For Registration to Ph.D. Degree in Botany.

Submitted by:-

Gobind Prasad

University Department of Botany

Binod Bihari Mahto Koyalanchal University, Dhanbad

Supervisor

Dr. Pallavi Praveen

Assistant Professor

Department of Botany

B.S.City College, Bokaro – 827006

Scholar

Gobind Prasad

Department of Biotechnology

B.S.City College, Bokaro- 827006

Studies of Phytochemical Screening and Estimation of Antimicrobial Activity of Medicinal Plant Extract *Achyranthes aspera* Linn.

1. INTRODUCTION:

Achyranthes aspera Linn. is a species of plant belonging to *Amaranthaceae* family, commonly known as Apamarga or Chirchita in Hindi. It is a popular medicinal plant which has a prominent position in folk medicine throughout India, especially in Jharkhand

It is an erect herb, about 60 cm high with stiff, cylindrical and hairy branches. The leaves are simple and opposite. Main root is long cylindrical and thick; secondary and tertiary roots are present which are slightly ribbed. Odour is slight, taste is slightly sweet and mucilaginous. Fruits are sharp pointed orange to reddish purple capsules. Seeds are sub cylindrical, truncates at apex, rounded at base, black and shining.

Achyranthes aspera is a very important plant for its large number of medicinal properties as well as many pharmacological activities like, anti-allergic, cardiovascular, nephroprotective, anti-parasitic, hypoglycemic, analgesic and antipyretic.[1]

This herb is reported to have various pharmacological actions such as anti-inflammatory, analgesic, and antipyretic activities. It is used to treat many problems such as piles, digestive disorders, fever (especially malarial fever), cough, dysentery, asthma, hypertension diabetes, paralysis, spleen enlargement, control of fertility, and postpartum bleeding. While some people also use the roots of this plant as tooth brush, it still has a long way to go to be established as a recognized entity in the prevention and treatment of oral diseases. [2]

Numerous non aromatic hydrocarbon compounds have been reported from the seeds and the shoots of *Achyranthes aspera*. The dried herb is used to treat children for colic and also as an astringent in gonorrhea treatment. Leaf extracts are reported to possess' hypoglycemic thyroid stimulating and antiperoxidative properties. [3]

Plant *Achyranthes aspera* has been classified as:-
Class – Magnoliopsida
Order – Caryophyllales
Family – Amaranthaceae
Genus – *Achyranthes*
Species – *aspera.*

PHYTOCHEMISTRY: *Achyranthes aspera* is traditionally valued as a potent medicinal agent. Chemical constituents like ecdysterone n-hexacos-17-enoic,spinasterol, achyranthine, betaine, pentatriacontane, 18-pentatriacontanone, hexatriacontane and tritriacontane , hydroquinone, P-benzoquinone, Spathulenol, nerol, asarone and essential fatty acids of various parts of the plant have been isolated and identified. [4]

2. REVIEW OF LITERATURE:

Extracts of the leaves and callus of this plant in various solvents have been reported to show antimicrobial activity. It has also been reported that methanolic extracts possess antimicrobial activity. [5] Studied show that the ethanol and chloroform extracts of the seeds of *A. aspera* show mild to moderate antibiotic activity against *Bacillus subtilis*, *E.coli*, and *Pseudomonas aeruginosa.* [6] The anti-carcinogenic activity by using ethyl acetate extracts of *A. aspera*, which showed high antibacterial activity against *Streptococcus mutans* than other solvent extracts. [7]

It has been reported that the activity of methanolic extract for different parts of the plant exhibited very high antioxidant activity which was closer to standard L - Ascorbic acid (94%). The activity of root was 90%, stem and inflorescences was 93% and leaf 87%. The antioxidant activities of different parts in methanolic extract were above 90% except for leaves and the order followed was: Inflorescences = Stem >Root > Leaf. The antibacterial activity of plant extracts in Hexane, Chloroform, Ethyl acetate and Methanol was against *Streptococcus aureus* and *E. coli.* The zone of inhibition recorded for the different parts of the plant in different extracts and highest antibacterial activity against *Streptococcus aureus* was recorded as 22 mm by the chloroform extract of root at 25 mg concentration and 18 mm by the methanol extract of root at 25 mg concentration against *E. coli.* [8]

There has been reports of an alkaloid achyranthine, amino acids, arginine, histidine, lysine, cystine, threonine, methionine, leucine, isoleucine, phenylalanine, tryptophan, valine and carbohydrate, arhamnopyranosyl, ß-D glucronopyranosyl, *ß*-D galactopyranosyl, galactose, xylose, rhamnose and glucose, large amount of potash, hormones, ecdysterone and inokosterone in this plant. The plant shows anti-bacterial, anti-inflammatory and abortifacient activity. *A. aspera* elevates thyroid hormone level and is also reported to have anticoagulant, anti-arthritic, anti-tumor and anti-hepatocarcinogenic activity. The plant is also reported to possess antidepressant, wound healing and blocking the detection of a pain or injurious stimulus by sensory neurons. [9]

Further by the same workers *in vitro* activity was tested by four solvent and the results suggested significant antioxidant activity of *Achyranthes aespera* leaf extract. The free radical scavenging activity of the extract was

concentration dependent and inhibitory concentration 50(IC50) was observed at a concentration of 62.24μg/ml for DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity and 68.32μg/ml for H_2O_2 free radical scavenging activity. The DPPH free radical and hydroxyl radical scavenging activity of *Achyranthes aespera* leaf extract was comparable to the standard ascorbic acid. Total antioxidant activity estimated by β- carotene bleaching assay was found to be significantly high in *Achyranthes aespera* leaf extract as compared to ascorbic acid. The reducing power of the plant extract increases with increasing concentration which shows that the antioxidant compounds are electron donors and can oxidize intermediates of lipid per oxidation process.

There has been studies on the antimicrobial activity of *Achyranthes aspera* using agar well diffusion and serial dilution method. The dental pathogens *Bacillus pumilus*, *Bacillus thuringiensis*, *Pseudomonas aeruoginosa*, *Acinetobacter junii* and *Enterococcus faecalis* were isolated and identified from dental caries and dental abscess respectively. Results of this study revealed the strong antibacterial potential of isolated compound from *Achyranthes aspera* against dental pathogens. [10]

Investigations on various extracts of petroleum ether, methanol, ethanol, ethyl acetate and chloroform of medicinal plant *Achyranthes aspera* for their antibacterial activities against multi drug resistant organisms such as *Pseudomonas aeruginosa, Proteus mirabilis and Enterococcus faecalis* have been done. The organic extracts of both the leaf and stem parts of the plants at a concentration of 5 mg/ml and their activities were measured by estimating zones of inhibition as produced by antibiotic sensitivity method on Mueller-Hinton agar. The result of this research support the use for further analysis in the treatment of infectious diseases such as urinary tract

infection caused by bacteria and has significant scope for antibacterial research. [11]

Aqueous and methanol stem extracts of the plant *Achyranthes aspera* were tested against the Gram Positive tooth bacteria by disc diffusion method [12] .The maximum zone of inhibition obtained is 7 mm at concentration 200 mg/ml for the methanol extract and 4 mm at same concentration i.e., 200 mg/ml for the aqueous extract. The zone of inhibition in mm decreases constantly with decrease in concentration for both aqueous and methanol stem extracts of the plant *Achyranthes aspera* and the minimum zone of inhibition in mm is obtained finally at concentration 25mg/ml for both methanol and aqueous stem extract that is 3 mm and 1 mm respectively The methanol extract of the plant *Achyranthes aspera* shows better zone of inhibition than aqueous extract against gram positive tooth bacteria.

3. PROBLEM TO BE INVESTIGATED:

- To prepare leaf and stem extracts for detection of presence or absence of phytochemicals.
- Isolation of chemical constituents from crude plant material.
- Purification of isolated compounds.
- Standardisation of extract based upon the isolated compounds by using various instrumental methods like using TLC (Thin Layer Chromatography), HPLC (High Performance Liquid Chromatography) etc.
- To validate the traditional use of *Achyranthes aspera* as an antimicrobial agent in treatment of oral infectious diseases.

4. OBJECTIVE OF THE RESEARCH:

The problems mentioned above will be studied with the following objectives-

1. To improve our understanding of the antimicrobial activity of *Achyranthes aespera*

2. To find out the exact nature of phytochemicals present in the plant extract.

5. HYPOTHESIS

The extract of different parts of *achyranthes aspera* have been reported to show antimicrobial activity in folk medicine but the identification of the particular active compounds and the exact mechanism of antimicrobial activity has not yet been conclusively established. So the present work will be aimed at finding out the exact nature of active compounds present in the plant and their antimicrobial activity.

6. RESEARCH METHODOLOGY:

I. Study area and Procurement of raw material

Achyranthes aspera leaves and stems will be collected from local area of Barki Dundi, Karma, District-Ramgarh (Jharkhand). After collection materials will be washed carefully and dried to constant weight at 45°C. The materials will be stored in an air-tight container.

II. Extraction process

Leaves and stem will be separated from plant to make powder separately by using mixer grinder. Powder parts will be separated with the help of soxhlet apparatus. Petroleum ether, chloroform, benzene, ethanol and ethyl acetate will be used sequentially .The extract will be then concentrated by successive cold and hot extraction of air dried *Achyranthes aspera* plant parts material using separation flask **(Cold and Hot Extraction Method**).

III. Test for anti-microbial activity

The bacterial strains *Staphylococcus aureus* and *Escherichia coli* species will be pre-cultured and cell density standardized spectrophotometrically. Extracts of different parts of *Achyranthes aspera* will be examined for antibacterial activities. After the incubation period they will be used as

inoculums and the standard procedure used to measure antibacterial activity and also anti-fungal activity will be screened by the use of **Disc- Diffusion Method**. The sterile extract disc will be prepared using filter paper Whatman No.1. It will be soaked in extracts of different concentrations. Bacterial strains and fungal strains will be spread on respective media. Test extracts load discs will be placed on bacterial lawn, fungal lawn and incubated on suitable temperature. After that incubation process zone of different inhibitions will be measured. Gentamycin will be used as standard disc for bacteria and ketoconazole as standard antibiotic for fungi will be used for comparing anti-microbial activity study of test sample. [13]

IV. Fractionation and separation

Eluted Fraction will be collected by using column chromatography and the fraction will be left for crystallization. After crystallization, filtration unit will be used for the separation of powder form of compounds.

V. Isolation and Characterization of compounds

The isolation of molecules from the plant extract will be done by using column chromatography and the isolated molecules will be characterized by spectral data.

VI. Standardization of isolated compounds.

Standardization of the extract will be carried out by using TLC (Thin Layer Chromatography), HPLC (High Performance Liquid Chromatography) etc. on the basis of isolated marker compounds.

VII. Statistical analysis

Statistical comparisons of mean value between the stem and leaf extract of different solution will be done by using student's t-test for statistical analysis.

7. SIGNIFICANCE OF PROPOSED WORKED:

Achyranthes aspera is one of the most widely distributed medicinal herbs found in our country. Hence the phytochemical analysis of these parts for its antimicrobial activities is of great relevance and significance. The aim of the study is to extract the alkaloids and anti-oxidant present in the medicinal plant *Achyranthes aspera* and examine their antimicrobial activity**.** It will help in to identifying the active compounds and to elucidate the exact mechanism of action by which they exert their biological effects. The unexplored areas of activities of alkaloids and antioxidants found and their antimicrobial activity will be investigated.

8. RESEARCH LIMITATION:

The different solvents use for extraction may not be able to dissolved all kinds of active compounds present in plant extract.

This may create difficulty in identification and accurate effectively of extract.

9. FUTURE PROSPECTS:

The phytochemicals and active compounds present in the plant which will be established as a result of present investigation can be used in finding out the exact mechanism of their antimicrobial activity. The pharmacological experiments performed during the present investigation can be extended to the next level of clinical trial to generate novel drugs.

10. TENTATIVE CHAPTERIZATION:

CHAPTER 1: INTRODUCTION:

This chapter will be an introduction to the numerous medicinal properties of the plant under investigation (*Achyranthes aspera*) and it's application in different pharmacological activities.

The curative properties of different chemical constituents of the plants on the basis of traditional as well contemporary medicinal system will also be introduced.

CHAPTER 2: REVIEW OF LITERATURE:

In this chapter the advancement made in the field related to the present investigation will be mentioned.

CHAPTER 3: STUDY AREA AND TAXONOMICAL DETAILS OF THE PLANT UNDER INVESTIGATION:

This chapter will include the detailed meteorological and geographical details of the area from which study sample will be collected and the taxonomical details of the plant under investigation.

CHAPTER 4: MATERIAL AND METHODS:

This chapter will include the detailed information about different methods used for extraction, test for antimicrobial activity, fractionation and separation, isolation and characterization of compounds and standardization of isolated compounds.

CHAPTER 5: PREPATION OF PLANT EXTRACTION AND TEST FOR ANTIMICROBIAL ACTIVITY:

This chapter will include the details of the extraction process of collected plant materials and its test for antimicrobial activity. It will also include fractionation, separation and characterization of active compounds.

CHAPTER 6: STANDARDIZATION OF PHYTOCHEMICALS AND SEPARATION OF PHYTOCHEMICALS:

In this chapter the methods used for standardization of the isolated compounds will be discussed in details and the separated phytochemicals will be reviewed.

CHAPTER 7: STATISTICAL ANALYSIS:

In this chapter data analysis will be done by using student t –test for statistical analysis of results obtained.

CHAPTER 8: RESULTS, DISCUSSION AND CONCLUSIONS.

This chapter will include the results obtained from various test methods which will be used, discussion of the results and conclusion on the basis of the nature of results.

REFERENCES

[1] Pandey, B., Bajpai, P., Singh, S., and Shrivastava, S. (2014). Study of physiochemical analysis of *Achyranthes aspera* extracts. *Int J Pharm Sci Res.* 5(8): 3378- 3382.

[2] Yadav, R., Rai, R., Yadav, A., Pahuja, M., Solanki, S., and Yadav, H. (2016). Evaluation of antimicrobial activity of *Achyranthes aespera* extract against *Streptococcus mutans*: An *in vitro* study. *J Adv pharm Technol Res.* 7(4): 149-152.

[3] Varuna, K. M., Khan, M. U., and Sharma, P. K. (2010). Review on *Achyranthes aspera*. *Journal of Pharmacy Research.* 3(4): 714-717.

[4] Lakshmi, V., Mahdi, A. A., Sharma, D. and Agarwal, K. S. (2018). An Overview of *Achyranthes aspera Linn.* , *Journal of Scientific and Innovative Research.* 7(1): 27-29.

[5] Prabhat, Ajaybhan, Navneet, and Chauhan, A. (2010). Evaluation of antimicrobial activity of six medicinal plants against dental pathogens. *Report and Opinion*. 2: 37–42.

[6] Khan, A. V., Ahmed, Q. U., Shukla, I., and Khan, A. A. (2012). Antibacterial activity of leaves extracts of *Trifolium alexandrinum Linn*. against pathogenic bacteria causing tropical diseases. *Asian Pac J Trop Biomed*. 2(3): 189-194.

[7] Jebashree, H. S., Kingsley, S. J., Sathish, E. S., and Devapriya, D. (2011). Antimicrobial activity of few medicinal plants against clinically isolated human cariogenic pathogens – An *in vitro* study. *ISRN Dent*. 541421.

[8] Santhi, J. R., Beaulah, A. G., and Sadiq, M. A**. (2011).** Antioxidant and antibacterial activity of *Achyranthes aspera* : An *in vitro* study. *Der Pharma Chemical.* 3 (5): 255-262.

[9] Pandey, G., Rao, C. V., Gupta S. S., Verma, K. K., and Singh, M. (2015). Antioxidant and antibacterial activities of leaf extract of *Achyranthes aspera Linn*, *European Journal of Medicinal Plants*. 4: 495-708.

[10] Patil, A. G., and Jobanputra, A. H. (2013). *In vitro* antimicrobial potential of an active phytoconstituent isolated from the ethanolic extract of *Achyranthes aspera* stem against oral pathogens. *International Journal of Advanced Life Sciences.* 6(5): 549-553.

[11] Pandey R, Sambasivarao, Y., and Gurumurthy (2013). Antibacterial activity of medicinal plants against pathogens from extracts of *Achyranthes aspera*. *Med Aromat Plants.* 2(5): 1-3.

[12] Panda, A., Sabat. G., and Mohanty, B. K. (2018). Study of antibacterial activity with stem extracts of *Achyranthes aspera, L., International Journal of Advanced Scientific Research and Management.* Volume 3 Issue 10.

[13] Parveen, S. (2018). Phytochemical screening and antimicrobial activity of herbal plant extracts- *Achyranthus aspera*, *International Journal of Pharmaceutical and Clinical Research.* 10 (7): 201-209.